Dynamic Changes in Striatal Dopamine D\(_2\) and D\(_3\) Receptor Protein and mRNA in Response to 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) Denervation in Baboons

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Loss of nigrostriatal neurons leads to striatal dopamine deficiency and subsequent development of parkinsonism. The effects of this denervation on D\(_2\)-like receptors in striatum remain unclear. Most studies have demonstrated increases in striatal dopamine D\(_2\)-like receptors in response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-mediated denervation, but others have found either decreases or no change in binding. To clarify the response to denervation, we have investigated the time-dependent changes in dopamine D\(_2\), D\(_3\), and D\(_4\) receptor protein and mRNA levels in unilaterally MPTP-lesioned baboons. MPTP (0.4 mg/kg) was infused into one internal carotid artery, producing a contralateral hemi-parkinsonian syndrome. After MPTP treatment, the animals were maintained for 17–480 d and then euthanized. MPTP decreased ipsilateral dopamine content by >90%, which did not change with time. Ipsilateral D\(_2\)-like receptor binding in caudate and putamen initially decreased then increased two- to sevenfold over the first 100 d and returned to near baseline levels by 480 d. Relative levels of D\(_2\) mRNA were essentially unchanged over this period. D\(_4\) mRNA was not detected. In contrast, D\(_3\) mRNA increased sixfold by 2 weeks and then decreased. At the peak period of increase in binding sites, all D\(_2\)-like receptors were in a micro-molar affinity agonist-binding state, implying an increase in uncoupled D\(_2\) but not D\(_3\) receptor protein. Taken together, these data suggest that MPTP-induced changes in D\(_2\)-like dopamine receptors are complex and include translational or post-translational mechanisms.

Key words: Parkinson’s disease; MPTP; dopamine D\(_2\) receptor; dopamine D\(_3\) receptor; caudate; putamen

Degeneration of nigrostriatal neurons produces striatal dopamine deficiency with the subsequent development of parkinsonism including bradykinesia, rigidity, resting tremor, and postural instability (Hornykiewicz, 1963; Wooten and Trugman, 1989). Initially, replacement therapy with levodopa replenishes striatal dopamine and ameliorates symptoms. However, as the disease progresses and treatment continues there is loss of the smooth clinical response to medication and the development of fluctuations in motor symptoms including dopa-induced involuntary movements. The pathophysiological bases of these clinical changes and the role of alterations in dopamine receptors in initial denervation and chronic treatment remain unclear. The discovery of the selective dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al., 1983; Ballard et al., 1985) has made possible the development of a model of dopaminergic degeneration and parkinsonism (Burns et al., 1983; Bankiewicz et al., 1986).

Despite a large number of MPTP animal studies associating clinical descriptive changes with exposure to different dopamine agonists and antagonists (Lau and Fung, 1986; Gagnon et al., 1990; Alexander, 1991; Calon et al., 1995), there have been few quantitative analyses of the underlying receptor mechanisms. Reports regarding the effects of MPTP on dopamine receptors are controversial. It is generally observed that there is little change in D\(_1\)-like receptor number. Some report a decrease in dopamine D\(_1\)-like receptors, whereas others report no change or an increase (Lau and Fung, 1986; Falardeau et al., 1988; Gagnon et al., 1990; Weihmuller et al., 1990; Alexander et al., 1991). It is now recognized that there are multiple dopamine receptors that are the products of separate genes (Grandy and Civelli, 1992). Hence, it is possible that changes in D\(_2\)-like receptor binding described in previous studies may be attributable to changes in the expression of a single receptor subtype or to complex changes in the expression of D\(_2\), D\(_3\), or D\(_4\) receptors.

Striatal dopamine D\(_2\)-like receptors include both presynaptic autoreceptors on afferents from dopamine-synthesizing cells and postsynaptic receptors. The principal striatal D\(_2\)-like receptor is D\(_2\) in both rat and human (Boundy et al., 1993; Levy et al., 1993; Fisher et al., 1994; Lahti et al., 1995). D\(_2\) receptors are expressed in striatum but at much lower levels than D\(_3\) (Boundy et al., 1993; Ariano and Sibley, 1994; Lahti et al., 1995). D\(_4\) receptors are absent or present in only small amounts (Seeman et al., 1993; Lahti et al., 1995; Mrzlajak et al., 1996). Transfection studies utilizing dopamine-producing cell lines have demonstrated that D\(_2\) and D\(_3\) receptors, but not D\(_4\) receptors, can function as autoreceptors (Tang et al., 1994a,b; O’Hara et al., 1996). Both D\(_2\) and D\(_3\) receptors can regulate dopamine synthesis and release. Whether striatal autoreceptors are principally D\(_2\) or D\(_3\) is unclear.

In the present study, we have developed a paradigm by which...
baboons can be unilaterally lesioned with MPTP, an approach first described for monkeys by Bankiewicz and colleagues (1986). This approach has the advantage that animals become hemi-parkinsonian after a single MPTP injection and can therefore be used without requiring any dopaminergic medication. Hence, the time course of changes in dopamine, receptor number, and mRNA can be followed without the potential confounding effects of medication intervention.

MATERIALS AND METHODS

All animal procedures including means to minimize discomfort were reviewed and approved by the Washington University Animal Studies Committee. Precautions were also taken to minimize investigator exposure to MPTP.

External carotid ligation. At least 4 weeks before the administration of MPTP (i.c.v.), we ligated one external carotid artery to decrease the amount of recirculating MPTP during the procedure described below. Animals were fasted overnight but allowed free access to water up to 2 hr before the procedure. Anesthesia was induced with ketamine (25 mg/kg, i.m.) and maintained with xylazine (2 mg/kg, i.m.), and secretions were reduced with atropine (0.04 mg/kg, i.m.). A 20-gauge plastic catheter was inserted into a limb vein for administration of medications, and a soft-cuffed endotracheal tube was placed to protect the airway. Ophthalmic ointment (Lacrilube) was put in each eye to protect the corneas. The animal was placed in the supine position, and the mandible was exposed. The area from the base of the skull to the shoulder was shaved, washed with alcohol and betadine, and draped. Anesthesia was induced with ketamine (10–15 mg/kg, i.v.), and the animal was maintained with thiopental (10–15 mg/kg, i.v.), and the animal was placed in the supine position, and the mandible was exposed. The common carotid and external carotid were identified and dissected with alcohol and betadine, and draped. A 6–8 cm skin incision in the neck was made beginning at the angle of the mandible using electrocautery. The common carotid and external carotid were identified and dissected free. The external carotid was doubly ligated close to the origin, and the wound was closed.

MPTP administration. For MPTP treatment, anesthesia was induced with ketamine (10–15 mg/kg, i.m.), atropine (0.04 mg/kg, i.m.) was given to reduce secretions, and an endotracheal tube was placed. Anesthesia was maintained with thiopental (10–15 mg/kg, i.v.), and the animal was then paralyzed with 2–4 mg/kg gallamine intravenously. A 20-gauge catheter was inserted percutaneously into a femoral artery, and then a 0.021 inch Teflon guide wire was inserted through that catheter. The catheter was removed, and a #5 French Hanafee catheter (80 cm long) slid over the guide wire and positioned into the common or internal carotid artery under fluoroscopic control. The position was confirmed by bolus injection of ~5 cm³ of contrast (RENO-76), and a radiograph documented this positioning. MPTP (0.4 mg/kg) dissolved in 30–50 ml of saline was infused into the carotid artery through a 22 gauge micropore filter over 30–45 min (not faster than 1–2 ml/min) (Bankiewicz et al., 1986; Guttman et al., 1990; Palombo et al., 1990). The Hanafee catheter then was removed and paralysis reversed with edrophonium (10 mg, i.v.). The animal was allowed to recover and was observed until it was able to drink water and care for itself. All animals were videotaped to document the response to MPTP.

Sample preparation and storage. Animals were euthanized by injection of pentobarbital (120 mg/kg, i.v.), and the brains were quickly removed and dissected (Perlmutter et al., 1991). Samples for dopamine levels, receptor binding studies, and mRNA measures were stored at ~70°C in sealed plastic pouches until further analysis.

In vitro analysis of tritiated spiperone binding sites. Membranes from caudate-putamen were prepared as described previously (Todd and Bauer, 1988) and assayed for tritiated spiperone (specific activity 23–25 Ci/mmol; DuPont NEN, Boston, MA) binding in the presence and absence of 1 μM eticlopride (Perlmutter et al., 1991). For saturation binding, membrane samples (40–150 μg of protein) were assayed in triplicate in a total volume of 1 ml that contained final concentrations of 120 mM NaCl, 50 mM Tris, pH 7.4, and 0.1–10.0 nM tritiated spiperone ± 1 μM eticlopride. Incubations were for 60 min at 20°C. Samples were diluted with cold buffer, rapidly collected onto glass fiber filters, and washed twice with cold buffer using a modified Brandel cell harvester. As demonstrated previously (Perlmutter et al., 1991), eticlopride-displaceable tritiated spiperone binding is saturable and reversible under these conditions. In control animals, there is no effect of the inclusion of up to 2 mM CaCl₂ or 4 mM MgCl₂ on spiperone binding. Because of its low affinity for eticlopride, D₂ receptor binding would not be detected under these conditions (Tang et al., 1994a). To distinguish D₂ and D₃ receptor binding, agonist binding was performed in the presence and absence of GTP analogs; membranes were incubated with 1 nM [³H]spiperone and increasing concentrations of 7-hydroxy-2-(di-n-propyl)-aminotetraline (7-OH-DPAT; 0.1 nM to 30 μM) in the presence or absence of 10 μM guanyl-5’-imidodiphosphate [Gpp(NH)p] as described previously (Tang et al., 1994a).

Saturating and competition binding variables were determined for membrane binding data using nonlinear curve-fitting programs as implemented by Lundon Software programs (Chagrin Falls, OH). Using these assays conditions, we have documented previously the lack of any significant right/left differences in tritiated spiperone binding in either the putamen or the caudate of the baboon (Perlmutter et al., 1991).

In vitro analysis of mRNA. The sequences of oligonucleotide primers for the detection of D₂, D₃, or D₄ mRNAs were derived from either rat or baboon genomic DNA sequences. In each case, the primer pairs for a given receptor crossed an exon boundary. The D₂ receptor primers o-197 and o-198 were as described by O’Malley et al. (1990). These generated an expected product size of 344 bp. The primer pair for the D₃ mRNA was o-198 and o-580 (Tang et al., 1994), with an expected product size of 350 bp. The primer pair for D₄ mRNA was o-513 and o-515 (O’Malley et al., 1992), with an expected product size of 242 bp. For detection of D₄ genomic DNA, the following primers were used: o-513 with o-542 (5’-CTCCGAGTAGACGAAGAG), complementary to nucleotides 389–406, accession number M84009; and oligonucleotide o-515 with o-522 (5’-CATGGGCAATGGAGCTCAT), complementary to nucleotides 448–465. These sets produced product sizes of 132 and 65 bp, respectively.

For amplification of RNA, 1 μg of total RNA (Chomczynski and Sacchi, 1987) was reverse-transcribed (Krug and Bergher, 1878) with an oligonucleotide complementary to the individual RNA (D₂: o-198; D₃: o-198; D₄: o-515). The resulting cDNAs were used for the PCR with the primers indicated above. Temperatures for the PCR protocols for individual receptor mRNAs were as follows: D₂: 93°C for 1 min, 61°C for 1 min, 72°C for 1 min, 28 cycles; D₃: 93°C for 1 min, 50°C for 1 min, 72°C for 1 min, 28 cycles; D₄: 93°C for 1 min, 50°C for 1 min, 72°C for 1 min, 30 cycles. All mRNA samples were standardized by using complementary oligonucleotide primer sets to the 18S fragment of rRNA (Chan et al., 1984) as described previously (Mack et al., 1991). For quantification, oligonucleotides were end-labeled and added to the PCR mixtures. PCR products were separated on 12% polyacrylamide gels, the gels were dried, and radioactivity was quantified using a Molecular Dynamics PhosphoImager (Molecular Probes, Eugene, OR) and software. Standard RNAs were included on all gels to allow comparisons of levels of expression between different experiments. For D₂ and D₃ receptor mRNA levels in baboon striatum, the levels detected at 28 PCR cycles were still within the exponential portion of the amplification curve (Mack et al., 1991).

Dopamine measurements. Dopamine levels in baboon brain tissue samples were determined by HPLC with electrochemical detection as described previously (Parkinson et al., 1981). Amounts of dopamine were determined by integration of peak heights relative to calibration standards and corrected for extraction efficiency by the internal standard ratio method. Tissue levels are expressed relative to the weight of tissue (ng/gm).

Statistical analysis. For multiple comparisons of binding data or mRNA levels, one-way ANOVA was used to estimate overall significance followed by post hoc t tests corrected for multiple comparisons by the method of Bonferroni (Miller, 1981). All data were normally distributed, and significance levels of t test comparisons were adjusted for inequality of variances when appropriate. All analyses were completed using the SAS suite of programs (SAS Institute, Cary, NC). These experiments were begun in 1987. The preparation and analysis techniques have remained unchanged to ensure compatibility of data across animals.

RESULTS

After the animals were euthanized, brain regions were dissected and frozen at ~70°C. Samples for dopamine content or mRNA levels were taken, and remaining caudate and putamen tissue from both sides of each brain were prepared for membrane binding assays. Samples were analyzed from five control animals and from single animals euthanized 17, 18, 38, 45, 101, 112, 262, and 480 d after unilateral MPTP lesioning. Equal numbers of animals were lesioned on the left and right sides.
Changes in dopamine content

Caudate and putamen samples from both sides were assayed for dopamine content in the same animals (Parkinson et al., 1981). The mean ± SE caudate and putamen dopamine content for five control animals was 8496 ± 1622 and 9148 ± 740 ng/gm tissue, respectively. There were no left/right concentration differences. After MPTP lesioning, there were no changes in contralateral dopamine content. Compared to contralateral tissues, MPTP lesioning resulted in a 95–100% decrease in ipsilateral caudate and putamen dopamine content at all but the last time point measured (17–480 d; Fig. 1). For the 480 d animal, the decrease was 91 and 99% for ipsilateral caudate and putamen, respectively. The mean ± SE caudate and putamen dopamine contents for the ipsilateral side of five MPTP-lesioned animals were 211 ± 87 and 197 ± 124 ng/gm tissue, respectively (p < 0.001 vs controls). The mean ± SE caudate and putamen dopamine contents for the contralateral side were 10291 ± 2641 and 9571 ± 1981 ng/gm tissue, respectively (no difference from control values).

Changes in receptor protein

Samples from both sides of each animal were analyzed for [3H]spiperone binding. As shown in Figure 1 for both caudate and putamen, initial decreases in D2-like binding at 17 and 18 d after MPTP lesioning were followed by significant increases in D2-like receptor binding that were first detected ~40 d after lesioning. Receptor number continued to increase until ~100 d after lesioning and then decreased to approximately control levels by 480 d. These highly significant changes in receptor number (ANOVA, p < 0.0001) were not accompanied by significant changes in the affinity of the receptors for the ligand (ANOVA, p > 0.1). For example, the Kd values ± SEM for ipsilateral putamen were 64 ± 10, 36 ± 15, 40 ± 23, 62 ± 36, 132 ± 104, 80 ± 25, 197 ± 115, 116 ± 44, and 25 ± 17 pm for 0–480 d after MPTP lesioning, respectively. For caudate, there were two- to threefold increases in receptor number on the lesioned side. There was also a parallel but smaller nonsignificant increase in receptor number on the noninjected side. The pattern of changes was somewhat different in putamen. First, there was a six- to eightfold increase in receptor number on the ipsilateral side by 100 d after MPTP lesioning. Second, there was also a large and significant increase in receptor number on the contralateral side (ANOVA, p < 0.001).

Contralateral changes in receptor binding suggest that there was either recirculation of injected MPTP to the other side of the brain or a bilateral effect from other transynaptically mediated mechanisms. Whatever the mechanism, these changes did not decrease contralateral dopamine content (see above) or result in a bilateral parkinsonian syndrome (Perlmutter, 1993). Furthermore, the decrease in ipsilateral and contralateral receptor number after long-term recovery from MPTP lesioning is not secondary to changes in dopamine content. This suggests that the mechanism for decreasing dopamine receptor number after 100 d is not attributable to reinnervation or enhanced synthesis of dopamine by surviving nigral neurons. These bilateral changes in receptor number demonstrate that the uninjected side cannot be used as a within animal control. The asymmetric clinical state of these animals, however, still makes this a useful model system for the analysis of MPTP-induced changes in the absence of other pharmacological treatment.

Detection of dopamine D2, D3, and D4 mRNA

The dopamine D2-like receptors have not been cloned from baboon. Hence, to detect mRNAs, probes were developed that are conserved across species. Synthetic oligonucleotides were designed that recognized human and rat dopamine D2, D3, and D4 mRNA. All of the described primer pairs cross an intron–exon junction to distinguish amplification products attributable to mRNA from those attributable to contaminating genomic DNA. Total cellular RNA was isolated from tissues and reverse-transcribed (RT) using a complementary oligonucleotide specific for the given receptor subtype, and the resulting cDNA was amplified by the PCR using a second oligonucleotide specific for each receptor type (RT-PCR). D2 and D3 primer pairs amplified the expected sized products for D2 and D3 mRNA from putamen (Fig. 2). The identification of D2 and D3 receptor sequences was confirmed by probing amplified sequences with an internal primer specific for each receptor type (data not shown). In contrast, even under low-stringency conditions using up to 60 cycles of PCR we could detect no D4 mRNA in either caudate or putamen (Fig. 2). To confirm that this D4 primer pair could recognize baboon sequences, baboon genomic DNA was amplified under conditions allowing the generation of long PCR products. The expected 1.7 kb product was detected (data not shown). As a further confirmation that the chosen primers would recognize baboon mRNA if present, each of these primers was paired with another D3-specific oligonucleotide that would not cross an intron–exon boundary. Baboon DNA was prepared and amplified with the two

Figure 1. Time course of changes in striatal D2-like receptor binding and dopamine content after MPTP lesioning. Animals were killed at the indicated times after unilateral MPTP administration, and samples were prepared for receptor number and dopamine content measurements as described in Materials and Methods. Values shown for receptor number are the mean ± SEM of two to four independent saturation binding experiments repeated in triplicate except for the putamen day 45 study in which tissue was only available for a single determination in triplicate. Samples from the injected (filled circles) and noninjected (open circles) regions were assayed simultaneously for each animal. The day 0 values are the mean ± SE of two to four independent saturation binding experiments repeated in triplicate for the same animals (Parkinson et al., 1981). Values shown for dopamine content (filled squares) represent the ratio of tissue values from the injected side divided by the noninjected side. Actual dopamine contents are described in the text.
new sets of D4 oligonucleotides. In each case, the expected sized product was detected in genomic DNA (Fig. 3). Thus, D2 and D3 mRNAs are easily detected in RNA prepared from control baboon striatum, whereas no D4 message is detectable.

**MPTP induced changes in mRNA levels**

Total RNA samples from ipsilateral putamen of MPTP-treated animals were reverse-transcribed with D2-, D3-, and D4-specific primers and subjected to amplification to determine specific receptor mRNA content. Under these conditions, the amount of product amplified was linearly related to the amount of input RNA. The same control samples were included on all gels to allow multiple individual experiments to be grouped together for analysis. In all cases, three to six individual determinations of mRNA content were conducted.

As shown in Figure 4, there was a modest increase in dopamine D2 mRNA expression after MPTP lesioning (ANOVA, \( p = 0.0013 \)). However, the only pairwise significant difference from control values was found for the day 101 animal (\( p = 0.017 \)).

Moreover, as shown by comparison with the changes in receptor number, there was no correlation between D2 mRNA level and receptor number (\( r = 0.37 \), not significant).

In contrast, there were marked changes in D3 mRNA levels as early as 17 d after MPTP lesioning (Fig. 2). D3 mRNA levels increased up to sixfold and then decreased to control levels.
Because of the sensitivity of this assay, we can conclude that affinity in the presence or absence of Gpp(NH)p (quinpirole competition was best described by a single binding site of low (Gpp(NH)p, the competition was best fit by a single class of binding sites analysis. solid lines components are displayed as solid lines in the figure. In the presence of Gpp(NH)p, the competition was best fit by a single class of binding sites (K_i = 4.2 μM; dashed line). For MPTP-treated putamen membranes, quinpirole competition was best described by a single binding site of low affinity in the presence or absence of Gpp(NH)p (filled circle, K_i = 1.5 μM; open circle, K_i = 0.6 μM). See Materials and Methods for details of data analysis.

(ANOVA, p < 0.0001). Compared to controls, levels for days 17, 18, 101, and 112 were two- to sixfold higher. As with D2 mRNA levels, there was no correlation between D3 mRNA levels and receptor number (r = -0.26, not significant). No D4 mRNA was detected at any time point.

The pattern of mRNA level changes is difficult to reconcile with the changes observed for dopamine D2-like receptor number. To test whether the early increase in D3 mRNA levels resulted in later increases in D3 receptor number, D2 and D3 receptor binding was differentiated at the day of peak binding (101 d after MPTP lesioning).

**Discrimination of D2 and D3 receptor binding**

The GTP insensitivity of agonist binding to D3 receptors was used to discriminate D2 and D3 receptors (Sokoloff et al., 1990; Seabrook et al., 1992; Boundy et al., 1993; Tang et al., 1994a; Malmberg and Mohell, 1995). As shown in Figure 5, in control animals 7-OH-DPAT binding to D2-like receptors is best described by a combination of high-affinity (nanomolar) and low-affinity (micromolar) sites. The mean ± SE K_i values (and percentage of sites) for agonist binding were 74 ± 5 nM (48 ± 9%) and 2.7 ± 0.9 μM (52 ± 9%), respectively (n = 3). In the presence of the nonhydrolyzable GTP analog Gpp(NH)p (10 μM), all high-affinity binding is shifted to a low-affinity state (K_i = 1.7 ± 0.7 μM, n = 5). Because of the sensitivity of this assay, we can conclude that <10% of the total eticlopride-displaceable [3H]spiperone binding sites are attributable to D1 receptors in untreated tissue. It should be noted that under these conditions no D4 binding could be detected (Tang et al., 1994a). A similar analysis was conducted on membranes from putamen of an animal that had been treated with MPTP for 101 d. Ipsilateral putamen from this animal expressed sevenfold higher binding sites than control tissue (Fig. 1). When agonist competition for [3H]spiperone binding was done in the presence or absence of 10 μM Gpp(NH)p, there was no evidence for high-affinity binding sites. The mean ± SE K_i values were 0.4 ± 0.1 μM (n = 3) and 1.1 ± 0.3 μM (n = 5) in the absence and presence of Gpp(NH)p, respectively. We conclude from these binding studies that there is no evidence for an increase in D3 receptor binding at this time point. Further, the existing D2-like binding sites are in a low-affinity state. These results are most compatible with MPTP-induced increases in D2-like binding secondary to an increase in D3 receptors that are in a low-affinity, uncoupled state.

**DISCUSSION**

MPTP lesioning is a useful model for studying basic mechanisms involved in denervation, reinnervation, and drug response. Here we show that after unilateral MPTP denervation of baboon striatum there are large and complex, time-dependent fluctuations of ipsilateral dopamine D2-like receptor binding that do not correlate with changes in the expression of D2, D4, or D3 mRNA levels or with dopamine content. The simplest interpretation of these findings is that the initial decrease in D2-like receptor binding at 17–18 d after MPTP lesioning is secondary to the destruction of dopamine autoreceptors from terminals of dopamine-synthesizing cells. The subsequent phases of a large increase until 100 d after MPTP lesioning and then decrease in D2-like receptor number are most likely secondary to post-transcriptional changes in D2 receptor translation, modification, or stabilization. Notably, agonist competition binding studies in the presence of GTP analogs suggest that the phase of increased D2-like receptor binding is attributable to a specific increase of low-affinity uncoupled D2 receptors (Fig. 5). The subsequent return to baseline levels of expression of total D2-like receptor binding with time occurs in the absence of changes of dopamine content, demonstrating that this is not secondary to reinnervation or enhanced synthesis of dopamine by remaining nigral dopamine-producing cells.

Our findings for control baboon striata are similar to those reported for rat and human tissue (Boundy et al., 1993; Levy et al., 1993; Seeman et al., 1993; Ariano and Sibley, 1994; Fisher et al., 1994; Lahti et al., 1995). Specifically, both D2 and D3 receptor mRNAs were easily detected whereas no D4 receptor mRNA was present (Fig. 2). Based on the effects of GTP analogs on agonist binding, the major expressed receptor was D2 in baboon striatum (Fig. 5). Because of the use of eticlopride to define nonspecific binding, D4 receptor binding was not directly assessed. D4 receptors are unlikely to be present, however, given the absence of D4 mRNA in striatum (Fig. 2) and the lack of evidence that the D4 receptor can serve as an autoreceptor. Taken together with the previous results of our comparison of in vivo and in vitro D2-like receptor binding (Perlmutter et al., 1991), we find no evidence for left/right differences in the concentrations of striatal dopamine, dopamine receptors, or dopamine receptor mRNAs.

The magnitude of the increases in [3H]spiperone binding in baboon striatum (300–800%) are larger than those reported after MPTP lesioning in other species. For studies showing an increase in [3H]spiperone binding, the reported increases for striatum are between 20 and 40% for rodents (Lau and Fung, 1986; Weihmuller et al., 1990) and 15–50% for monkeys (Farlardeau et al., 1988;
Gagnon et al., 1990). Using unilateral lesioning of rat nigra by 6-hydroxydopamine, Fornaretto et al. (1993) also observed a time-dependent increase in ipsilateral [H]piperidine binding of 25% in striatum by 90 d that decreased to control levels by 365 d. Qin et al. (1994) reported a 10–20% increase in ipsilateral D2-like receptor binding and mRNA ~4 weeks after unilateral 6-hydroxydopamine lesioning of mouse striatum. 

Despite much literature on the effects of MPTP on dopaminergic systems, there are few studies examining the relationship between dopamine loss and receptor density over time. Using a bilateral MPTP treatment paradigm (Burns et al., 1983), Farlardeau et al. (1988) demonstrated significant increases in monkey dopamine receptor number only if dopamine depletion was at least 90% of control values. In this study, however, animals were euthanized 1–5 months after the injection of MPTP. Hence, time-dependent changes in receptor number complicate the interpretation of this finding. The current study as well as the previous report by Weihmuller et al. (1990) using a unilateral MPTP treatment paradigm with mice indicate that complex changes in D2-like receptor binding occur as a function of time. Weihmuller et al. (1990) found a 33% decrease in D2-like binding 3 d after cessation of MPTP treatment, an increase in binding of 40% over the next 2 months, and then a return to control levels of binding by 4–5 months. At 3–5 months after MPTP exposure, an increase in D1-like binding was observed. In contrast to the present study, however, in the mouse paradigm there was a return to pretreatment dopamine levels by 4 months after MPTP treatment (Weihmuller et al., 1989). Hence, in the rodent model the decrease in D2-like and increase in D1-like receptor number at long time periods may be the consequence of reinnervation or enhanced synthesis by dopamine-synthesizing neurons. In the current study, there was no recovery of dopamine content with time. As described above, Fornaretto et al. (1993) also observed an increase then decrease in binding in striatum in the absence of recovery of dopamine levels in 6-hydroxydopamine lesioned rats.

There was no correlation between changes in receptor protein and mRNA. A modest increase in dopamine D2 mRNA after MPTP was observed. However, this increase neither preceded the increase in receptor number nor correlated with receptor number overall. The significance of this observation, therefore, must be questioned. D2 mRNA was not detected at any time point. In contrast, there were marked early changes in D3 mRNA expression that remain elevated until the final experimental time point of 480 d. The initial increase in D3 mRNA was associated with the initial decrease in D2-like receptor binding, which is presumably secondary to the loss of terminal dopamine autoreceptors after destruction of dopamine-synthesizing cells. D3 mRNA levels remain elevated at later time points but were not associated with any evidence for an increase in D3 receptor number. It is possible that earlier changes in D3 receptor number were missed by restricting agonist binding studies to the 101 d time point of peak D2-like receptor expression (Fig. 5). It is also possible that the initial decrease in D2-like receptor number observed at days 17 and 18 is secondary to the specific loss of D3 autoreceptors. Unfortunately, insufficient tissue is available from these animals to address either of these questions. Qin et al. (1994) reported 10–20% ipsilateral increases in D2-like binding and D2 mRNA several weeks after 6-hydroxydopamine lesioning of mouse striatum. The time course of these changes, however, was not reported.

This study has several limitations. Because of the prolonged data-gathering period, it was not possible to incorporate technical advances such as newer receptor ligands or absolute mRNA number measurements into the design of the study. Also, the original choice of eticlopride to define nonspecific binding precluded the direct assessment of D2 receptors. Given our findings, however, it is unlikely that use of newer compounds or techniques would have changed our conclusions. Additionally, our choice of using membrane homogenates for receptor binding analyses resulted in a loss of information on receptor changes in specific regions of the caudate and putamen. Alexander et al. (1991) have reported that bilaterally MPTP-treated monkeys show larger changes in receptor number in the lateral caudate and the lateral putamen. Hence, we may be underestimating the magnitude of changes in either receptor number or mRNA level in subregions of the striatum.

Finally, it is of interest to note that the observed changes in receptor number after unilateral MPTP were bilaterally equal in the putamen. To our knowledge, the six- to eightfold increase in receptor number observed 101 d after MPTP treatment in putamen is the largest that has been reported. Although there were modest increases in caudate receptor number on the noninjected side as well, the bilateral increases in putamen receptor number were essentially indistinguishable at all time points examined. This suggests that receptors in baboon putamen may be more sensitive to MPTP or that there are other yet to be discovered trans-synaptic mechanisms that regulate putamen receptor levels bilaterally. Whatever the mechanism, it is not a simple dependence on local levels of dopamine per se because contralateral dopamine concentrations were the same as for control animals.

The current study demonstrates that changes in dopamine D2-like receptor binding after denervation are not simply related to changes in dopamine content or receptor mRNA metabolism. Moreover, unilateral lesioning results in a similar temporal pattern of changes in receptor number bilaterally. In future studies, it will be important to address whether similar temporal patterns of changes occur for D1-like receptors, nondopamine striatal receptors (such as serotonin), and related peptide transmitters. Electrophysiological analysis of bilateral striatal activity will help define the functional significance of the observed changes. Lesioning of the corpus collicus also may define the pathway(s) mediating bilateral changes in receptor concentration.

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